MICROMON SANGER SEQUENCING

PREPARATION OF DNA TEMPLATE FOR CYCLE SEQUENCING

The quality of the template DNA is one of the most critical factors in Sanger sequencing. Also important is that the template is at the correct concentration, is free of contaminants, dissolved in ultrapure water, and is not degraded.

Template quality and amount is important because these will ultimately determine the quality of the output basecalls (sequence resolution). A reduction in final resolution and signal intensity can be caused by the presence of RNA, salt, protein and other contaminating chemicals. These contaminants can be eliminated by effective template purification prior to cycle sequencing and a thorough post-reaction clean-up. These are both key factors in the generation of long, highly resolved reads.

At Micromon, we require that **ALL** template DNA to be used in the cycle sequencing reactions submitted to our facility, be post-extraction purified to eliminate the contaminants that cause instrument capillary degradation and reaction failure. Our requirement is that you use one of the many standard, commercially available column- or magnetic bead-based, DNA extraction/purification kits that are appropriate for the type of template you are using. We can provide a list of commonly-used products and suppliers if needed.

1. PLASMID **Mini-prep or maxi-prep**

The critical issue is to remove any contamination with chromosomal DNA, excess RNA or other cellular components. We can suggest a number of popular and commonly-used extraction and purification products and suppliers if requested. The only non-column or bead-based chemical method of plasmid extraction that is acceptable at Micromon Genomics is the AB modified alkaline lysis-PEG precipitation method (see user guides). Crude bacterial extracts, either from a liquid culture or a plate colony, must NEVER be used directly in cycle sequencing reactions for our facility.

2. PCR Products

There are two options, depending on the complexity of the PCR reaction. If the PCR product is unique, you can use ultra-filtration, magnetic beads, or a standard PCR purification column to remove the reaction components. Alternatively, the PCR products can be separated on an agarose gel and the desired DNA band, excised and retrieved. It should be noted that even with this method, it is still possible to excise extraneous PCR products of a similar size to the desired DNA. All traces of the original PCR primers need to be removed, as these could produce undesired bands by acting as primers in the cycle sequencing reaction.

3. Template DNA Elution from Purification Columns

It is essential that elution from the DNA extraction kits is carried out with either MilliQ-grade water or 1 mM TRIS solution but not TE buffer. The EDTA in TE buffer can inhibit the Taq DNA Polymerase in the cycle sequencing reaction by removing the Magnesium ions which are critical for the activity of the enzyme.

4. Template DNA Quantitation

To achieve an optimal sequencing data you must quantitate your template DNA solution. The use of spectrophotometry (usually with a Nanodrop or DeNovix Spectrophotometer) will generally not give a true determination of the template DNA concentration due to the remaining presence of low levels of contaminating RNA, chromosomal DNA, proteins, etc. Chromosomal DNA shows up as a smear on an agarose gel, following digestion. The optimal methodology is to use visualisation of the stained DNA on an agarose gel against a standard of similar size and known concentration. If your standard is linear then you must also linearise your plasmid for this determination. Once experienced, reasonable estimates can be made without the use of a standard.